Supporting information for

High throughput monitoring of bacterial cell density in nanoliter droplets: label-free detection of unmodified Gram-positive and Gram-negative bacteria

Natalia Pacocha[†1], Jakub Bogusławski[†2], Michał Horka[1], Karol Makuch[1,3], Kamil Lizewski[2], Maciej Wojtkowski[2], Piotr Garstecki*[1]

[1]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

[2]International Centre for Translational Eye Research, Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, Poland

[2]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

[†] equal contribution

*Corresponding author:
E-mail: garst@ichf.edu.pl

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Schemes of microfluidic chips for droplet generation and reading

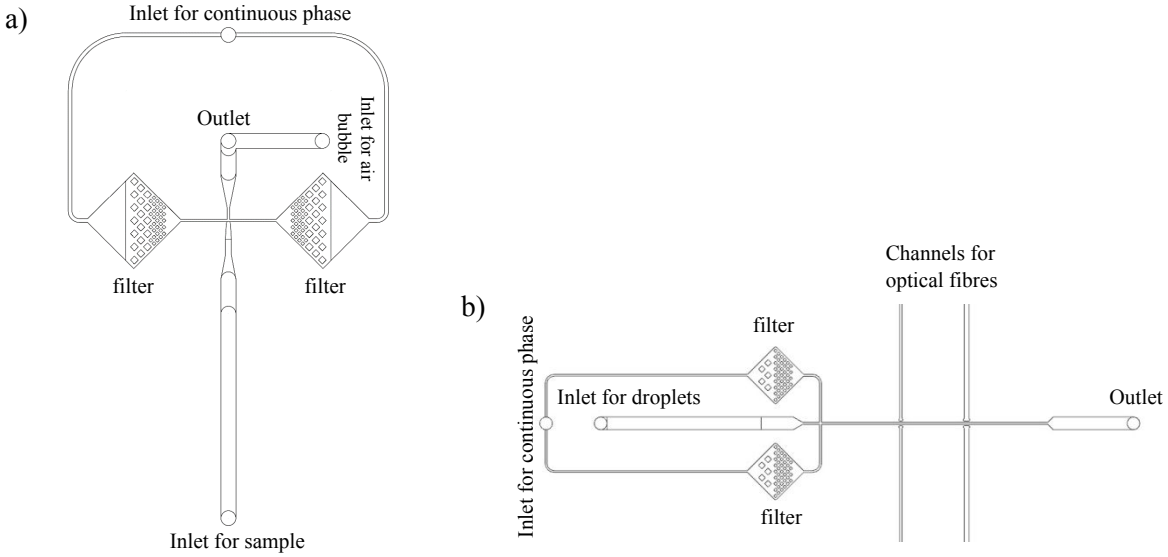

Figure S1. Schematics of a) a chip for droplets generation, b) a chip for droplets reading.

The dimensions of a chip for droplet generation are following:

- channel for disperse phase at inlet, width 800 µm x height 800 µm,
- channel for continuous phase, width 200 µm x height 200 µm,
- flow-focusing junction, width 100 µm x height 120 µm,
- outlet channel, width 800 µm x height 800 µm.

The dimensions of a chip for droplet reading are following:

- channel for droplets, width 800 µm x height 100-1200 µm,
- channel for continuous phase, width 120 µm x height 100 µm,
- flow-focusing junction, width 120 µm x height 100 µm,
- detection channel, width 120 µm x height 100 µm.

Exemplary droplet signals recorded in scattering channel

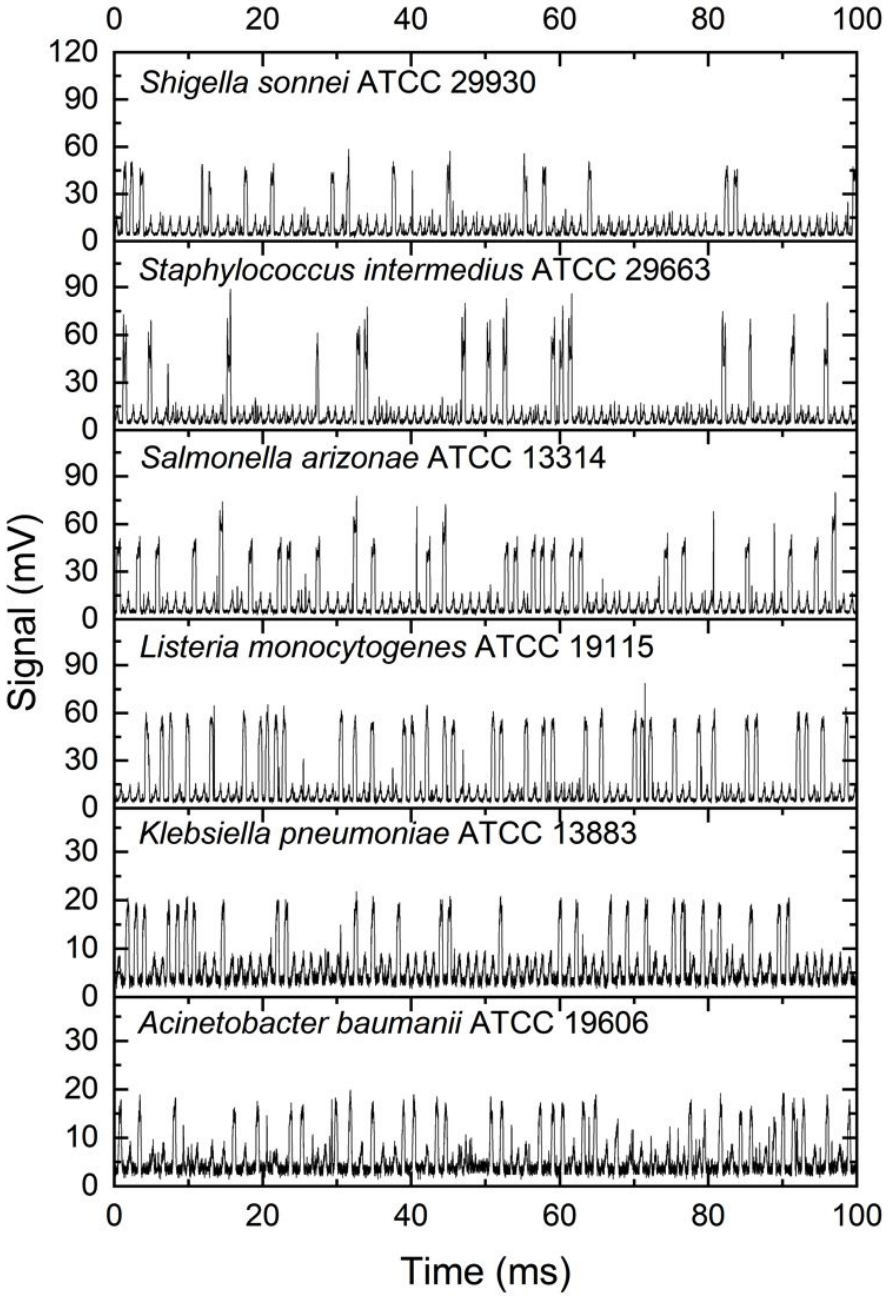

Figure S2. Exemplary waveforms of six unlabeled samples consisting of 70% of negative droplets (culture medium) and 30% of droplets containing high concentration of tested strain of bacteria.

Numerical analysis of data towards heterogeneity

Droplets are formed through process of emulsification of a suspension of bacteria. We assume that it leads to a random number of bacteria inside droplet according to the Poisson distribution: probability that there will be $\boldsymbol{n}$ bacteria inside droplet if given by the following equation:

$$p_n = \frac{\langle N_{CFU} \rangle^n}{n!} e^{-\langle N_{CFU} \rangle}. \quad (1)$$

It contains the average number of bacteria in a droplet $\langle N_{CFU} \rangle$, that is equal to the product of the number density of bacteria in a suspension before incubation and of droplet volume, $\langle \mathrm{N_{CFU}} \rangle = \mathrm{n_b V}$. Initially a nonempty droplet - after incubation without antibiotic - will contain a big number of bacteria. It will give a positive signal in our detectors. In presence of antibiotic with concentration c we assume that a bacteria will grow with probability

$$F_R(c) = \int_c^\infty dmp(m). \quad (2)$$

Here $\boldsymbol{F_R(c)}$ is the fraction of resistant bacteria in population that will grow in droplets with antibiotic concentration c. Therefore, $p(c)$ is the probability density that a bacteria will grow in the concentration c or lower. Equivalently, $p(c)$ is the distribution of scMIC of bacteria in the population. Without antibiotic all bacteria will grow, $\int_0^\infty dmp(m) = 1$.

In our model, we assume that two (or more) bacteria incubated in the same droplet will grow independently. We confine ourselves to the situation with small number of bacteria inside droplet: most of droplets are empty, about 10% of droplets contain a single bacteria, and there is a small fraction of droplets with two and more bacteria. For this reason, the assumption of independent growth is not crucial in our considerations. The above model of independent bacteria growth and their Poissonian distribution allow us to calculate probability that a droplet after incubation will contain many bacteria (will give a positive signal),

$$p_{bp}(c) = 1 - e^{-\langle N_{CFU} \rangle \int_c^\infty dmp(m)}. \quad (3)$$

In our experiments we observe false positives. Even for the highest concentrations of antibiotic we detect a small fraction of positive droplets. We also observe that the fraction of false positives does not depend on the antibiotic concentration. False positives appear also for the case of samples without bacteria. Therefore we assume that a positive signal appears independently on antibiotic and bacteria presence. We denote probability that a droplet will lead to a false positives by p_{fp}. Because the probability of a false positive is independent on the presence of bacteria, the positive signal may appear independently with probability p_{fp} and $p_{bp}(c)$. Therefore, the probability of a positive is given by

$$p_+(c) = p_{bp}(c) + p_{fp} - p_{bp}(c)p_{fp}. \quad (4)$$

In our experiment we have N droplets. N_+ droplets among N give a positive signal. We define fraction of positive droplets,

$$f_+(c) = \frac{N_+}{N} \quad (5)$$

and repeating measurements several times we obtain its variance $\sigma_{f_+(c)}$. Because fraction of positive droplets is equal to probability that a droplet give a positive signal, $\boldsymbol{f}_+(\boldsymbol{c}) = p_+(c)$, in this way we also determine $\boldsymbol{p}_+(\boldsymbol{c})$ and its error.

We observe in our experimental data that for a few highest concentrations of antibiotic there is a consistent value of fraction of positives. In these cases concentrations are sufficiently large to assume that bacteria do not grow, $\boldsymbol{p_{bp}(c)} = 0$. Utilizing it in formulas (3) and (4) we obtain, $p_{fp} = f_+(c_{large})$ with c_{large} denoting any of such high concentrations. In our calculations we use the highest available concentration. In this way we determine p_{fp} with its error, $\sigma_{p_{fp}} = \sigma_{f_+(c)}$. We then use it in formulas (1) and (2) for zero antibiotic concentration obtaining expression for the average number of bacteria

$$\langle N_{CFU} \rangle = -\log \frac{1 - p_+(0)}{1 - p_{fp}}. \quad (6)$$

Using the above equation along with (1) and (2) we get the formula for fraction of resistant bacteria

$$F_R(c) = \log \frac{1 - p_+(c)}{1 - p_{fp}} / \log \frac{1 - p_+(0)}{1 - p_{fp}}. \quad (7)$$

Error of the fraction $F_R(c)$ is calculated from the error propagation of the above formula.